SOMATIC STORAGE OF *PROTOSTRONGYLUS* SPP. THIRD STAGE LARVAE IN BIGHORN SHEEP

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Abstract:

Examination of tissue digest from bighorn sheep (*Ovis canadensis canadensis*) ewes demonstrates that the lung is a site of somatic storage of *Protostrongylus* spp. third stage larvae (L_3). L_3 of *Protostrongylus* spp. were recovered from lungs of 14 of 17 (82.4 percent) ewes originating in or obtained from three areas in Colorado. L_3 were not recovered from any other tissue examined. Ewes less than 1 year old were not infected.

INTRODUCTION

Virtually all of the bighorn sheep (*Ovis canadensis canadensis*) in Colorado are infected with lungworms in the genus *Protostrongylus*. Previous investigators suggested prenatal infection in lambs by *Protostrongylus* spp. (Pillmore 1956, Rufi 1961, Forrester and Senger 1964, Howe 1965). More recently, Hibler et al. (1972, 1974) showed that transplacental infection of lambs occurred during the third trimester of pregnancy. They demonstrated third stage larvae (L_3) of *Protostrongylus* spp. in the cotyledons of ewes and the liver and lungs of fetal and neonatal lambs.

Theoretically, ewes ingest L_3 with the snail intermediary host during the spring, summer, and fall months and store some of these L_3 somewhere in the body (somatic storage) until the third trimester of pregnancy; stored L_3 are then transmitted to the fetus. Knowledge of the site(s) of somatic storage may yield information regarding immunology of the parasite; moreover, effects of anthelminthics against L_3 may be better evaluated if the storage site(s) is known.

MATERIALS AND METHODS

Seventeen ewes dying from various causes were necropsied from late August 1973 through late January 1975. Following necropsy their tissues were prepared and examined using a modification of the technique described by Baermann (1917). Each tissue was ground or minced and placed in 3.7 l jars containing water for 24 hours to allow any larvae present to exit. Ground tissue was removed with a strainer and then washed several times to remove any larvae remaining on tissue surfaces. This tissue was held for

digestion. Washings were allowed to settle for 30 minutes to permit any larvae present to sink to the bottom. Excess fluid was then decanted. Sediment and the remaining fluid were examined for larvae. Tissues were then placed in 3.7 l jars and a Pepsin-HCl solution was added to digest tissues, allowing any larvae still trapped to be released. After 24 hours of digestion, sufficient formaldehyde was added to provide a 10 percent formalin solution and stop digestion; this also preserved any larvae present. Once again, the decanting procedure was followed. Since the sediment remaining was very thick, anhydrous ether was added (20 percent, v/v) to float the digested organic matter, leaving lungworm larvae in the aqueous layer below. The organic layer was removed by aspiration with a pipette and rubber bulb. The aqueous layer was examined for larvae. Specific tissues examined for L_3 were: skeletal muscle including muscle from the four limbs, facial, cervical, sublumbar, supralumbar, thoracic area and abdomen; diaphragm, soft and hard palates, tongue, gums, salivary glands, trachea, pharynx, nasal turbinates, sinus scrapings, lungs, esophagus, rumen, reticulum, omasum, abomasum, small and large intestine, pancreas, liver, gallbladder, spleen, peritoneum, omentum, thyroid, thymus, kidneys and ureters, urinary bladder adrenals, ovaries, uterus, vaginal tissue, mammary glands, body fat, heart, pericardium, major arteries and veins, lymph nodes, brain, spinal cord, eyes, integument and bone marrow.

RESULTS AND DISCUSSION

Protostrongylus spp. L_3 were recovered from the lungs and the reproductive tract of 14 of 17 (82 percent) ewes. The results, including other pertinent data are given in Table 1. Captive sheep were not re-exposed to L_3. The lungs of the seven pregnant ewes (41 percent of the total 17 examined) accounted for 368 L_3 (69 percent) of 542 L_3 recovered. Since L_3 were not recovered from the yearling ewe (#16) possibly the young do not store larvae. The Baermann technique with modification is still a poor one at best to recover L_3 from tissues. Some tissues do not digest as well as others, e.g. lung digests very well while trachea digests poorly. Also the great mass of tissue to be examined in this way allows room for human error to occur. For these reasons L_3 stored in other somatic tissues may not have been found and not all L_3 stored in the lungs may have been recovered.

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TABLE 1

Data of Animals Examined for *Protostrongylus* spp. L_3

Case	Origin	Age*	Date Died	*Protostrongylus* spp. L_3 Recovered: Lungs	*Protostrongylus* spp. L_3 Recovered: Reproductive Tract and Fetus	Comments
1	†Pikes Peak, Colo.	A	Aug. 1973	1	0	Captive 8 months
2	†Pikes Peak, Colo.	A	Aug. 1973	1	0	Captive 8 months
3	†Pikes Peak, Colo.	A	Nov. 1973	0	0	Captive 8 months
4	†Pikes Peak, Colo.	A	Nov. 1973	2	0	Captive 10 months
5	†Pikes Peak, Colo.	A	Nov. 1973	2	0	Captive 10 months
6	Pikes Peak, Colo.	A	Feb. 1974	124	0	2-4 weeks pregnant
7	Pikes Peak, Colo.	A	Feb. 1974	55	0	Not pregnant, 10+ years old
8	Pikes Peak, Colo.	A	Feb. 1974	124	0	8-10 weeks pregnant
9	†Pikes Peak, Colo.	A	Mar. 1974	34	0	Aborted fetus, 14-16 weeks pregnant
10	†Pikes Peak, Colo.	A	Mar. 1974	20	0	Aborted fetus, captive 3 days
11	†Pikes Peak, Colo.	A	Mar. 1974	23	3	Aborted fetus, 14-16 weeks pregnant, captive 3 days
12	†Pikes Peak, Colo.	A	Mar. 1974	20	4	Aborted fetus, 18-20 weeks pregnant, captive 2 weeks
13	†Pikes Peak, Colo.	A	Mar. 1974	23	6	Small fetus
14	†Pikes Peak, Colo.	A	Apr. 1974	0	0	Captive since 1969 at Denver Zoo
15	Poudre Can., Colo.	L	Jan. 1975	0	0	Found nearly dead
16	Poudre Can., Colo.	Y	Jan. 1975	4	0	Captive 6 days
17	Mt. Evans, Colo.	A	Jan. 1975	19	0	Found dead

* A = adult
Y = yearling
L = lamb

† These animals had been treated with various anthelminthics. Drug efficacy will be discussed in a later publication.

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